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GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF DIMEDONE DERIVATIVES OF FORMALDEHYDE AND OTHER ALIPHATIC ALDEHYDES ON CAPILLARY COLUMNS

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SUMMARY

The quantitative determination of small amounts of aliphatic aldehydes (formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde) in biological samples is difficult by the classical methods. The dimedone derivatives of these aldehydes were prepared and proved to be stable under the conditions of gas chromatography. The thermal properties of these derivatives were studied by thermal gravimetry, differential scanning calorimetry and thermal emission analysis, and the gas chromatographic peaks were identified by mass spectrometry. An analytical method was developed for the separation and determination of the dimedone adducts of aliphatic aldehydes by gas chromatography using capillary columns.

INTRODUCTION

Recent research indicates that there is a relationship between hypermethylation in biological systems and malignant cell proliferation[1,2]. The hypermethylation may occur in nucleic acids, proteins (*e.g.* histones) and other cell components. The mechanism can be either enzymatic transmethylation or direct chemical methylatione. In the latter reaction the most probable reactant is formaldehyde, which can be formed among other products from nitrosamines by the effect of N-demethylase enzyme. N-demethylase activity may increase in the presence of aromatic hydrocarbons[3] or as a result of viral infections[4].

According to earlier evidence, formaldehyde is a strong methylating agent[5]. It is also supposed that in several living organisms, formaldehyde from different sources plays a very important role in spontaneous methylation of various molecules (*e.g.*, nucleic acids, proteins, and ethanolamines).

The amount of formaldehyde in biological systems is usually small, so determination by classical methods is often difficult. We have developed a gas chromatographic (GC) method for the determination of dimedone adducts of formaldehyde and its higher homologues. The structure of the dimedone adducts was examined by mass spectrometry (MS) and the thermal stability by thermal investigations.

EXPERIMENTAL

Preparation of samples

To a solution or suspension of the aliphatic aldehyde in 5% acetic acid, an excess of dimedone was added with stirring. The mixture was kept at 80° overnight, then the crystals were separated and repeatedly recrystallized from ethanol until a constant melting point was reached (Table I).

TABLE I

EVAPORATION CHARACTERISTICS OF DIMEDONE ADDUCTS

Adduct	*Melting point (°C)*		*Points of evaporation (°C)*		
	Measured	*Ref. 6*	*Start*	*Maximum*	*Stop*
Dimedone	149–150	149–150	100	155	200
Formaldomedone	190	189–190	100	190	220
Acetaldomedone	141	140–141	110	180	250
Propionaldomedone	155–156	154–156	130	185	250
Butyraldomedone	134–135	134–135	140	190	250

Gas chromatography with packed column

GC analysis was carried out on a Packard series 7400 gas chromatograph, equipped with a flame ionization detector (FID). The 60 cm × 4 mm I.D. glass column was packed with 1.5% SE-30 on silylated Chromosorb G (60–80 mesh). Nitrogen was used as carrier gas at a flow-rate of 50 ml/min. The detector and injector block temperatures were 220°. The column temperature was changed from 100 to 210° after a 2-min initial hold, at a rate of 10°/min.

Capillary column gas chromatography

A Chromatron GCHF-18 gas chromatograph equipped with a FID was used with a 25 m × 0.25 mm I.D. glass capillary column. The internal surface of the column was graphitized and coated with SE-30 and SP-2401 (1:1). The carrier gas was nitrogen at a flow-rate of 1.8 ml/min. The injector temperature was 320° and the column temperature 270°.

Capillary column gas chromatography–mass spectrometry

A JEOL-made double focusing mass spectrometer (JMS-O1SG-2) was used in direct and GC–MS measurements. The ion source of the mass spectrometer was an electron impact one.

The experimental conditions were as follows: ionizing current, 200 μA: ionizing voltage, 75 eV; accelerating voltage, 10 kV; electrostatic analyser voltage,

1 kV; detector voltage, 1.6–2.5 kV (changable), pressure in ion source, 10^{-6} torr; pressure in the analyser, 10^{7} torr; mass range, 15–500; resolution power, 2000; sample introduction, with solid sampler or through GC. The data were processed by an on-line JMA-0231/0241 type data system.

The coupling between the capillary tube and the ion source was made by a platinum tube, 0.1 mm I.D. The tube was also thermostated at 200°. The capillary column for GC–MS was a 25 m × 0.25 mm I.D. glass capillary column coated with SE-30 (WCOT).

Thermal analysis

A DuPont thermal emission analyser was used, equipped with FID. The detector temperature was 520°. The carrier gas was nitrogen at a flow-rate of 30 ml/min. DuPont systems were also used for thermal gravimetry (TG) and differential scanning calorimetry (DSC).

RESULTS AND DISCUSSION

The structures of the dimedone adducts were established by MS. The mass spectra show (Table II) that they all have similar structures, *i.e.* each aliphatic aldehyde is bonded to two dimedone molecules. These adducts were analysed by GC on packed columns, but under the applied conditions not all of them gave distinct and identifiable peaks (Fig. 1). In case of acetaldomedone and propionaldomedone a few small peaks appeared beside the main peaks, suggesting that the adducts may have decomposed at the temperature of the injection. In order to test the thermal stability of the adducts, we analysed them by TG, DSC and thermal emission analysis (TEA). Table I shows the measured evaporation characteristics of dimedone adducts.

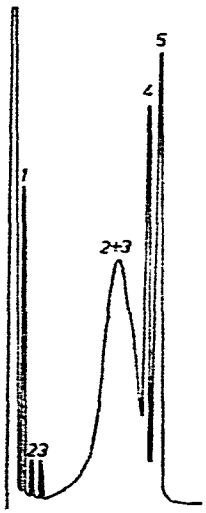

Fig. 1. Chromatogram of dimedone adducts. Column, 60 cm × 4 mm I.D. glass packed with 1.5% SE-30 on silylated Chromosorb G; detector, FID; carrier gas, nitrogen (50 ml/min); injector temperature, 320°; column temperature, 270°. Peaks: 1 = dimedone; 2 = acetaldomedone; 3 = propionaldomedone; 4 = formaldomedone; 5 = butyraldomedone.

TABLE II

CHARACTERISTIC MASS SPECTRA OF DIMEDONE DERIVATIVES

Dimedone		*Formaldomedone*		*Acetaldomedone*		*Propionaldomedone*		*Butyraldomedone*	
m/z	*Rel. int. (%)*	*m/z*	*Rel. int. (%)*	*m/z*	*Rel. int. (%)*	*m/z*	*Rel. int. (%)*	*m/z*	*Rel. int. (%)*
141	2.7	292 (*M*)	12.4	307	21.1	321	17.8	334 (*M*)	21.1
140 (*M*)	33.8	291	62.6	308 (*M*)	100.0	320 (*M*)	69.5	333	91.5
125	3.4	277	9.5	291	17.9	305	24.7	305	18.9
112	12.8	207	16.2	207	10.7	292	20.0	304	100.0
97	13.7	193	11.3	194	33.2	291	100.0	291	28.2
84	10.6	191	9.6	180	11.6	193	36.7	290	100.0
83	100.0	180	29.3	179	71.6	181	38.6	206	25.9
70	18.2	167	10.0	168	7.4	180	15.2	195	29.9
56	62.7	166	18.7	166	45.3	164	14.7	193	19.7
55	57.8	165	100.0	139	15.9	154	18.5	165	24.4
53	12.7	137	11.0	138	34.1	141	16.8	153	18.9
44	14.7	125	17.9	112	10.1	96	7.6	141	20.6
43	29.9	124	44.0	111	15.5	83	64.8	97	20.4
41	50.8	112	7.9	91	16.4	55	18.0	83	98.4
		111	8.2	83	49.8	44	20.1	55	48.7
		110	9.2	82	19.7	41	15.0	44	40.9
		97	24.3	69	15.5			42	41.6
		83	43.3	57	20.6				
		82	35.1	56	19.1				
		69	34.1	55	40.4				
		56	23.2	44	44.4				
		55	61.1						
		44	51.3						
		42	79.8						

The thermal data unambiguously show that all of these compounds sublimate and are not thermolabile under the GC conditions. Fig. 2 shows the TEA curves. Fig. 2 and Table I show that the dimedone adducts evaporate over a fairly wide temperature range, so their quick, quantitative evaporation can be accomplished only above 220–250°.

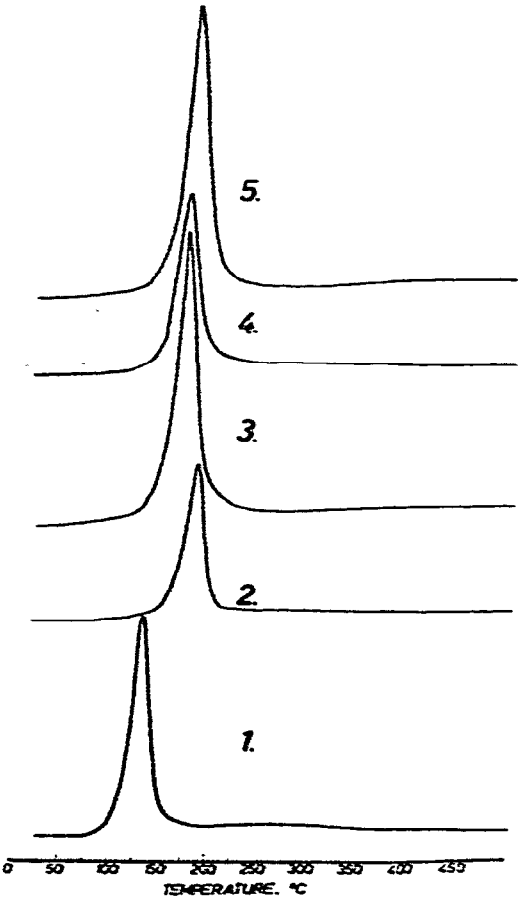

Fig. 2. Thermal emission analysis curves. 1 = dimedone; 2 = formaldomedone; 3 = acetaldomedone; 4 = propionaldomedone; 5 = butyraldomedone.

When GC–MS was carried out using capillary columns only single peaks appeared, indicating that the compounds do not decompose when evaporated. The mass spectra were identical with those of the pure original samples. The difficulties of the GC analysis of the adducts on packed columns may be explained by the possible tautomeric rearrangements of these compounds, which cause different inter- and intra-molecular interactions resulting in several peaks. Because of these problems we later used capillary columns, and determined the experimental conditions under which the five dimedone derivatives could be separated at the same time without decomposition, giving distinct peaks (Fig. 3). The retention values of the adducts

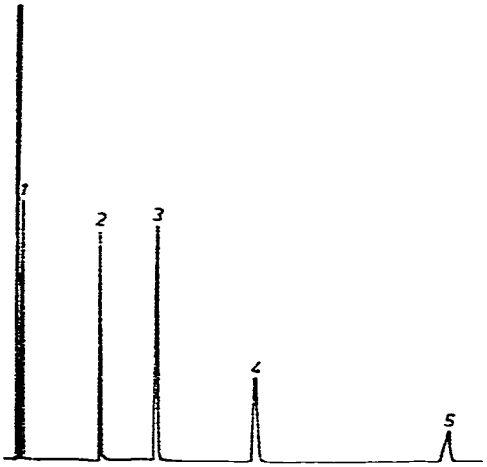

Fig. 3. Chromatogram showing the elution of dimedone adducts on capillary column. Column, 25 m × 0.25 mm I.D. glass capillary, graphitized, coated with SE-30 and SP 2401 (1:1); detector, FID; carrier gas, nitrogen (1.8 ml/min); injector temperatures, 320°; column temperature, 270°. Peaks: 1 = dimedone; 2 = formaldomedone; 3 = acetaldomedone; 4 = propionaldomedone; 5 = butyraldomedone.

gave a linear correlation with the number of the carbon atoms, which is an additional evidence that the adducts do not decompose under these conditions.

Our capillary column GC method is suitable for the identification and determination of these compounds in samples of biological origin.

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